ISSN 2278–5469
EISSN 2278–5450

Discovery

Proteomics analysis between cytoplasmic male sterility and restorer lines in Sunflower (Helianthus. annuus L)

Shabani Alireza

Esfahan, Payamenoor University Esfahan, Iran: Email: info_alireza2012@yahoo.com

Publication History
Received: 27 January 2017
Accepted: 7 March 2017
Published: 1 April 2017

Citation
Shabani Alireza. Proteomics analysis between cytoplasmic male sterility and restorer lines in Sunflower (Helianthus. annuus L). *Discovery*, 2017, 53(256), 266-271

ABSTRACT

In order to comparison between two cytoplasmic male sterile and fertile lines in sunflower *(Helianthus. annuus)*, a reference map of the major soluble proteins of sunflower seed was established using a combination of 2-DE and MALDI TOF and a total of 215 protein spots were detected with silver staining in a pH ranges of 3–10, of which 152 proteins were identified. These identified proteins were grouped into diverse functional categories. To further get an insight into the molecular basis of sunflower heterosis, differential proteome analysis between hybrid and parents were performed. A total of 7 differentially expressed protein spots were detected, and both quantitative and qualitative differences could be observed. Moreover 7 differentially expressed protein spots which identified were involved in metabolism, signal transduction, energy, cell growth and division, disease and defense, secondary metabolism. These results indicated that hybridization between two parental lines can cause expression differences between sunflower hybrid and its parents not only at mRNA levels but also at protein abundances.

Keywords: Differentially expressed protein, Heterosis, Mass Spectrometry, Two-dimensional gel electrophoresis, sunflower seed, MALDI-TOF.

1. INTRODUCTION

Seeds of higher plants serve many important functions, including a protective covering, a small embryonic and a nutrient-storage organ. Although the functional importance of seeds has been well recognized, and the molecular mechanism of seed development is still an area to be elucidated. Although the functional importance of seeds has been well recognized, and several genes associated with seed development have been characterized, the molecular mechanism of seed development is still an area to be elucidated. Proteomic studies of plant seed have contributed to our understanding of seed tissue differentiation and development in response to internal growth regulators as well as environmental signals [1]. sunflower hybrid exhibits the advantage of performance over its parents[2]. Attempts have been also made to characterize differentially expressed genes in seed between a hybrid and its parents, which revealed that differentially expressed genes represent diverse functional categories, such as metabolism, cell growth and maintenance, signal transduction, response to stress, transcription regulation and others [18, 20]. These results indicated that the hybridization between two parental lines can cause expression changes of different genes, which might be responsible for the observed heterosis [18–20]. However, changes on the level of mRNAs do not necessarily indicate changes on the protein level and/or in the hybrid phenotype, thus studies are needed to investigate differential proteomes between hybrids and its parents, and determine their functional relations to heterosis. In this paper, by using high-throughput 2-DE, a reference proteome map of sunflower seed with 215 Protein spots was constructed, and 152 proteins were identified. In addition,7 differentially expressed protein spots were detected in the seed proteomes between sunflower hybrid and its parents.

2. MATERIALS AND METHODS

Plant materials

Sunflower female line (CMS) obtained from the cross between Helianthus petiolaris (PET1) and H.argophyllus (ARG1). RHA280 registered No PI 413180 were used as fertility restoration (Rf) inbred lines. The materials have been generated in National Institute of Genetics and Biotechnology, NIGEB, Iran.

Total protein extraction and quantization

Total protein was isolated from seed tissues using Total protein was isolated from seeds using Invitrogen's TRIZOL® Reagent according to the manufacturer's instruction. Protein concentration was determined by Bradford assay.

2-DE and image analysis

The sunflower proteins in the dried powder were solubilized in 7 M urea, 2 M thiourea, 2% CHAPS (powder to solution/v), 0.5% pH 3–10 IPG buffer v/v (GE Healthcare, USA) and 36 mM 1,4-dithio-DL-threitol (DTT) (5.6 mg/ml) via incubationat room temperature for 1h, vortexing every 10 min, followed the mixture was centrifuged (15 000 rpm) for15 min, and the supernatant collected. Total protein extract(66 µl) was loaded onto GE Healthcare 18 cm IPG gel strips(pH 3–10L) during strip rehydration overnight, after which IEF was performed for a total of 65 kV.h using IPG Phor II(GE Healthcare) at 20C. The IEF buffer contained 7M urea,2 M thiourea, 2% CHAPS (powder to solution, w/v), 0.5%pH 3–10 IPG buffer v/v (GE Healthcare), and 36 mM DTT(5.6 mg/mL). The IPG strips were equilibrated according to manufacturer (GE Healthcare). PAGE gels (12% linear gradient) were run on an Ettan Dalt six (GE Healthcare), 0.5 h at 2.5 W *per* gel, then at 15 W *per* gel until the dye front reached the gel bottom. Upon electrophoresis, the protein spots were stained with silver nitrate according to the instruction of protein PlusOne™ Silver Staining Kit (GE Healthcare), which offered improved compatibility with subsequent mass spectrometric analysis. Briefly, gels were fixed in 40% ethanol and 10% acetic acid for 30 min, and then sensitized with 30% ethanol, 0.2% sodium thiosulfate w/v, and 6.8% sodium acetate w/v for30 min. Then gels were rinsed with distilled water three times, 5 min for each time, then incubated in silver nitrate(2.5 g/L) for 20 min. Incubated gels were rinsed with distilled water and developed in a solution of sodium carbonate(25 g/L) with formaldehyde (37%, w/v) added (300 mL/L)before use. Development was stopped with 1.46% EDTANa2.2.H_2O w/v, and gels were stored in distilled water until they could be processed and the reproducible spots removed from them. Gel images were acquired using Lab scan (GE

Healthcare). Image analysis was carried out with Image-master 2D Platinum Software Version 5.0 (GE Healthcare). Spot detection was performed with the parameters smooth, minimum area, and saliency set to 2,15, and 8, respectively, and was done automated by the software used, followed by manual spot editing, such as artificial spot deletion, spot splitting, and merging. All the gels were matched to the reference gel selected in automated mode followed by manual pair correction. The volume of each spot from three replicate gels was normalized against total spot volume, quantified, and subjected to ANOVA test (p,0.05). Spots of varied intensities were excised manually.

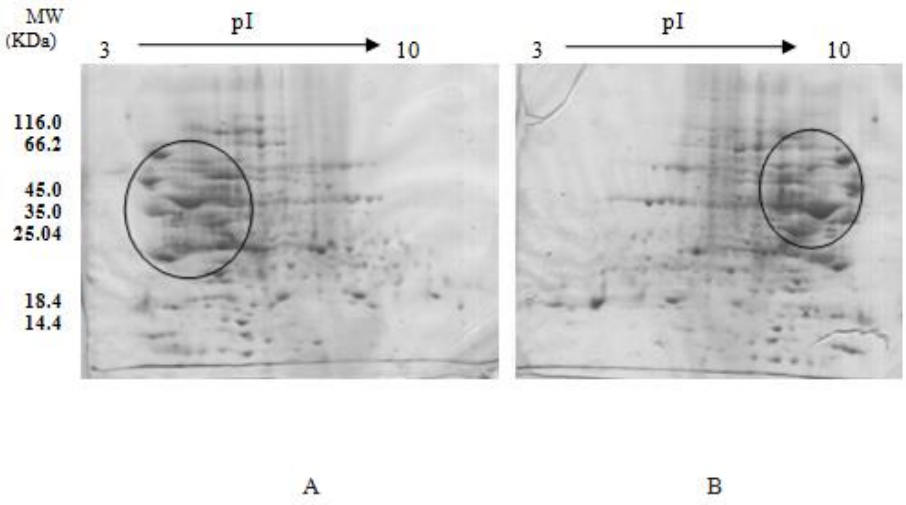

Figure 1

A comparison of protein profiles of sunflower fertile restorer inbred line (A) and cytoplasmic male sterile line (B) in 2-DE gels. Circles show major similarity spot pattern.

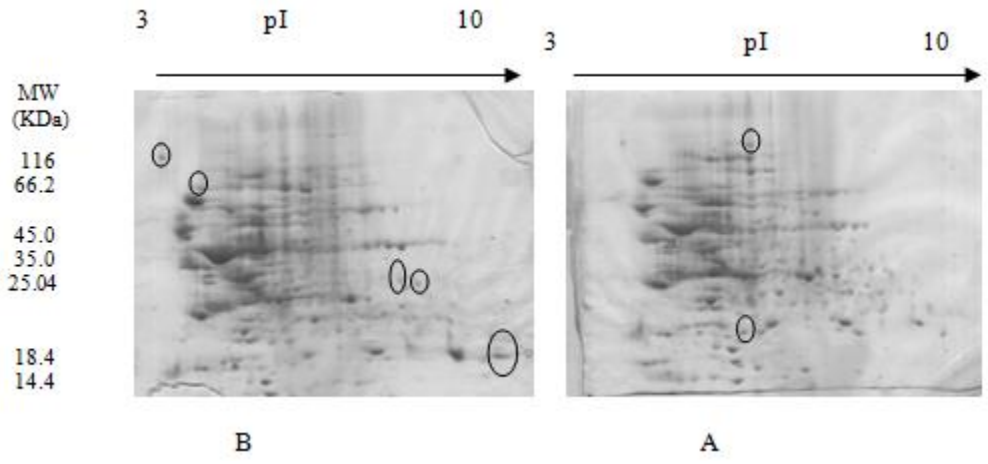

Figure 2

A comparison of protein profiles of sunflower cytoplasmic male sterile line (A) and fertile restorer inbred line (B) in 2-DE gels. Circles show differential spot pattern.

Shabani Alireza,
Proteomics analysis between cytoplasmic male sterility and restorer lines in Sunflower (Helianthus. annuus L),
Discovery, 2017, 53(256), 266-271,

3. RESULTS

Protein extraction is critical step for high-resolution 2-DE.Therefore, we tested several total proteins extraction procedures (TCA/acetone, phenol, and TRIZOL), and found that the method provided by TRIZOL Reagent (Invitrogen) produced the most clearly resolved gel. In addition, this method allows for the sequential extraction of RNA, DNA, and protein from the same tissue, which can be used for later investigation. formed on a linear gradient: pH 3–10. After IEF, proteins were separated according to their M_r in a second-dimension and stained with Coomassie Brilliant Blue. Using Image master 2D Platinum Software (GE healthcare), a total of 215 spots with M_r varied from 10 to 110 kDa, were reproducibly detected across three replicate gels from the sunflower female inbred line CMS and Restorer line RHA280 (Fig. 1).

2-D gel was employed to characterize the proteome expression profiles in seed of sunflower hybrid and its parents. The resolved protein spots on all the three replicate gels of hybrid and parents were analyzed by using Image master 2D Platinum Software (GE Healthcare). In total, 7 of 215 protein spots showed an accumulation difference of at least factor 1.5 between hybrid and parents and the differences of protein spots were also statistically significant by Student's *t*-test at $p \leq 5\%$, however, seven protein spots which showed presence/absence difference between the two parents and shown significant position changes between hybrid and its parents (as shown in Fig. 2 in Supporting Information) were not statistically analyzed. comparing the patterns of differentially expressed protein spots between hybrid and its parents represented that both quantitative and qualitative differences could be observed (Fig. 2).

The quantitative differences can be clustered into four categories:(i) up-regulated in hybrid (URH), expression in hybrid is higher than in both female and male parents;(ii) down-regulated in hybrid (DRH), expression in hybrid is lower than in two parents; (iii) high-dominant in hybrid (HDH), expression in hybrid is equal to the highly expressed parent; and (iv) low-dominant in hybrid (LDH), expression in hybrid is equal to the lowly expressed parent. Among the 45 differentially expressed protein spots, the number of spots in this study, the differential expression was observed mostly in qualitative differences expression in hybrid of protein only expressed either in paternal or maternal parents (Fig.2). These differentially expressed protein spots between sunflower hybrid and its parental lines were eluted from representative 2-D gels for identification, and spots were successfully identified. According to criteria used previously [24]

4. DISCUSSION

In this study, a reference proteome map of sunflower seed with 215 protein spots was constructed by using female inbred line HA 89 line which would improve our understanding of the specialization of this organ, especially in terms of physiological variation. The difference in the displayed protein spots number could be due to the differences in developmental stage, seed proteins classified in metabolism were largely related to enzymes of amino acid biosynthesis and catabolism, and carbohydrate metabolism and most of these enzymes showed great redundancy. This might reflect the importance of these processes in sunflower seed and the fact that many of these proteins are encoded by multigene families or frequently PTMs and is encouraging for future proteomics analysis of the dissection of seed functions. The main function of is energy harvesting, and storage through photosynthesis. Proteins involved in these functions are the most abundant seed proteins. Therefore, it is not surprising that a larger proportion of abundant proteins in the seed proteome are involved in energy metabolism [23]. One reason for the under-representation of signaling and regulatory proteins in our study could be that these proteins are usually low in abundance and might not be detectable on the gels, or they generated weak mass spectra that precluded identification [12]. In our study, 29% of the proteins were classified as unknown function . This discrepancy could be the result of differences of protein identification and database search in the two studies. However, it was also possible that more unknown proteins specifically accumulated in sunflower seed were detected in our study. Implication of differential proteome expression between hybrid and its parents in seed heterosis Studies indicated that gene expression differed between hybrids and their parents in sunflower, which may be responsible for hybrid vigor [18, 19, 36]. More recently, genome-wide analysis revealed that approximately of genes (1187 out of 8808) exhibited differential expression in seed by using the same hybrid and its parents as this study [20]. However, these studies were restricted to the expression analysis at mRNA level. In this paper, 2-DE was firstly employed to detect differential proteome expression of seed between hybrid and its parents in sunflower, and 3.2% protein spots (7 out of 215) showed accumulation difference, which was similar to the expression difference of 13.5% at transcriptional level. Taken together, our observation at translational level adds circumstantial evidence that expression differences between sunflower hybrid and its parents exist not only at mRNA levels but also at protein abundances. Moreover, based

on our present data, that is differentially expressed protein spots between sunflower hybrid and its parents exhibited dominance, it can be concluded that dominance could be the major molecular basis of seed heterosis.

ACKNOWLEDGEMENT

The author acknowledged the support from national institute of genetic engineering and biotechnology (NIGEB),in Iran For providing the necessary research facilities.

REFERENCES

1. Miernyk, Ján A., Hajduch, Martin. Journal of proteomics. 2011, 74, 389-400.
2. Basra, A.S.,. Heterosis and hybrid seed production in sunflower. In: Heterosis and hybrid seed production in agronomic crops. Eds. Virupakshappa, K. and Ranganatha, A.R.G. Food Products Press, An imprint of the Haworth Press Inc., New York, USA 1999, pp. 185-215.
3. Ueda, M., Koshino-Kimura, Y., Okada, K., Curr. Opin. Plant Biol. 2005, 8, 71–76.
4. Weijers, D., Benkova, E., Jager, K. E., Schlereth, A. etal.,EMBO J. 2005, 24, 1874–1885.
5. Okushima, Y., Overvoorde, P. J., Arima, K., Alonso, J. M. etal., Plant Cell 2005, 17, 444–63.
6. Guo, H. S., Xie, Q., Fei, J.F., Chua, N. H., Plant Cell 2005, 17,1376–1386.
7. Montiel, G., Gantet, P., Jay-Allemand, C., Breton, C., Plant Physiol. 2004, 136, 3478–3485.
8. Fizames, C., Munis, S., Cazettes, C., Nacry, P. etal., Plant Physiol. 2004, 134, 67–80.
9. Poroyko, V., Hejlek, L. G., Spollen, W.G., Springer, G. K. etal., Plant Physiol. 2005,138,1700–1710.
10. Ekman, D. R., Lorenz, W.W., Przybyla, A.E., Wolfe, N.L., Dean, J.F.D., Plant Physiol. 2003, 133, 1397–1406.
11. Kohler, A., Delaruelle, C., Martin, D., Encelot, N., Martin, F.,FEBS Lett.2003, 542, 37–41.
12. Koller, A., Washburn, M.P., Lange, B. M., Andon, N. L. et al.,Proc. Natl. Acad. Sci. USA 2002, 99, 11969–11974.
13. Hochholdinger, F., Woll, K., Guo, L., Schnable, P.S., Proteomics 2005, 5, 4885–4893.
14. Mathesius, U., Keijzers, G., Natera, S.H., Weinman, J.J. etal., Proteomics 2001, 1, 1424–1440.
15. Liu, Y., Lamkemeyer, T., Jakob, A., Mi, G.H., Zhang, F.S., Proteomics. 2006, 6, 4300–4308.
16. Sauer, M., Jakob, A., Nordheim, A., Hochholdinger, F., Proteomics.2006, 6, 2530–2541.
17. Sheffield, J., Taylor, N., Fauquet, C., Chen, S.X., Proteomics.2006, 6, 1588–1598.
18. Chang, W.W., Huang, L., Shen,M., Webster, C. etal., Plant
19. Bahrman, N., Gouy, A., Devienne-Barret, F., Hirel, B. etal.,Plant Sci. 2005, 168, 81–87.
20. Yao, Y.Y., Ni, Z.F., Zhang, Y.H., Chen, Y. etal., Plant Mol.Biol. 2005, 58, 367–384.
21. Wang, Z.K., Ni, Z.F., Wu, H.L., Nie, X.L., Sun, Q. X., Theor.Appl. Genet. 2006, 113, 1283–1294.
22. Zhang, Y.H., Ni, Z.F., Yao, Y.Y., Zhao, J., Sun, Q.X., Prog.Nat. Sci. 2006, 16, 712–720.
23. Broughton, W.J., Dilworth, M.J., Biochem. J. 1971, 125, 1075–1080.
24. Donnelly, B.E., Madden, R.D., Ayoubi, P., Porter, D.R., Dillwith, J.W., Proteomics 2005, 5, 1624–1633.
25. Porubleva, L., Velden, K. V., Kothari, S., Oliver, D. J., Chitnis, P. R., Electrophoresis 2001, 22, 1724–1738.
26. Bevan, M., Bancroft, I., Bent, E., Love, K. etal., Nature 1998,391, 485–488.
27. Bottley, A., Xia, G.M., Koebner, R.M., Plant J. 2006, 47, 897–906.
28. Gottlieb, L.D., Heredity.2003, 91, 91–92.
29. Mooney, B.P., Miernyk, J.A., Greenlief, C.M., Thelen, J. J.,Physiol. Plant. 2006, 128, 237–250.
30. Schwender, J., Goffman, F., Ohlrogge, J. B., Hill, Y.S., Nature.2004, 432, 779–782.
31. Giavalisco, P., Nordhoff, E., Kreitler, T., Klöpell, K.D. et al., Proteomics. 2005, 5, 1902–1913.
32. Li, H.P., Fischer, R., Liao, Y.C., Can., J. Plant Pathol. 2001, 23,286–291.
33. Persans,M.W.,Wang, J., Schuler,M. A., Plant Physiol. 2001,125, 1126–1138.
34. Austin, M.J., Muskett, P., Kahn, K., Feys, B. J. etal., Science.2002, 295, 2077–2080.
35. Horling, F., Lamkemeyer, P., König, J., Finkemeier, I. etal., Plant Physiol. 2003, 131, 317–325.
36. Chou, W.C., Huang, Y.W., Tsay, S.W., Chiang, T.Y. etal., Plant Physiol. 2004, 121, 50–57.
37. Bunney, T.D., van, Walraven., H.S., de Boer, A.H., PNAS.2001, 98, 4249–4254.
38. Wu, L.M., Ni, Z.F., Meng, F.R., Lin, Z., Sun, Q. X., Mol. Gen.Genomics 2003, 270, 281–286.
39. Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T., Lander, E. S., Genetics 1992, 132, 823–839.
40. Xiao, J., Li, J., Yuan, L., Tanksley, S.D., Genetics. 1995, 140, 745–754.

41. Yu, S., Li, J.X., Xu, C.G., Tan, Y.F. etal., Maroof, M.A.S., Proc. Natl. Acad. Sci.USA 1997, 94, 9226–9231.
42. Hua, J.P., Xing, Y.Z., Wu, W.R., Xu, C.G. etal., Proc. Natl.Acad. Sci. USA 2003, 100, 2574–2579.
43. Swanson-Wagner, R.A., Jia, Y., DeCook, R., Borsuk, L. A. etal., PNAS 2006, 103, 6805–6810.
44. Cheng, N.H., Gao, Y.P., Yang, J.S., Acta Bot. Sin. 1997, 39, 379–382.
45. Sun, Q.X., Wu, L.M., Ni, Z.F., Meng, F.R. etal., Plant Sci.2004, 166, 651–657.
46. Leonardi, A., Damerval,C., De Vienne, D., Genet. Res. Camb.1987, 50, 1–5.
47. Romagnoli, S., Maddaloni, M., Livini, C., Motto, M., Theor.Appl. Genet. 1990, 80, 769–775.
48. Bao, Y.Q., Kost, B., Chua, N.H., Plant J. 2001, 28, 145–157.
49. Clapham, D.E., Cell 1995, 80, 259–268.
50. Corbett, E.F., Michalak, M., Trends Biochem. Sci. 2000, 25, 307–311.
51. Llewelyn, R.H., Llewelyn, D.H., Campbell, A.K., Kendall, J.M., Cell Calcium 1998, 24, 253–262.
52. Corbett, E.F., Oikawa, K., Francois, P., Tessier, D. C. etal., J.Biol. Chem. 1999, 274, 6203–6211.
53. Opas,M., Szewczenko-Pawlikowski, M., Jass, G.H., Mesaeli, N., Michalak, M., J. Cell Biol. 1996, 135, 1–11.
54. Michalak, M., Corbett, E. F.,Mesaeli, N., Nakamura, K., Opas,M., Biochem. J. 1999, 344, 281–292.

Shabani Alireza,
Proteomics analysis between cytoplasmic male sterility and restorer lines in Sunflower (Helianthus. annuus L),
Discovery, 2017, 53(256), 266-271,